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Cloning of the Mouse Hepatitis Virus (MHV) Receptor: Expression in Human and Hamster Cell Lines Confers Susceptibility to MHV

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The cellular receptor for murine coronavirus mouse hepatitis virus (MHV)-A59 is a member of the carcinoembryonic antigen (CEA) family of glycoproteins in the immunoglobulin superfamily. We isolated a cDNA clone ($MHVR_1$) encoding the MHV receptor. The sequence of this clone predicts a 424-amino-acid glycoprotein with four immunoglobulinlike domains, a transmembrane domain, and a short intracytoplasmic tail. $MHVR_1$ is closely related to the murine CEA-related clone $mmCGM_1$ (*Mus musculus* carcinoembryonic antigen gene family member). Western blot (immunoblot) analysis performed with antireceptor antibodies detected a glycoprotein of 120 kDa in BHK cells stably transfected with $MHVR_1$. This corresponds to the size of the MHV receptor expressed in mouse intestine and liver. Human and hamster fibroblasts transfected with $MHVR_1$ became susceptible to infection with MHV-A59. Like MHV-susceptible mouse fibroblasts, the $MHVR_1$-transfected human and hamster cells were protected from MHV infection by pretreatment with monoclonal antireceptor antibody CC1. Thus, the 110- to 120-kDa CEA-related glycoprotein encoded by $MHVR_1$ is a functional receptor for murine coronavirus MHV-A59.

Coronaviruses are large, enveloped, plus-strand-RNA viruses which show marked tropism for epithelial cells of the enteric or respiratory tracts of their natural hosts. The murine coronaviruses, comprising numerous strains of mouse hepatitis virus (MHV), cause respiratory infection, inapparent enteric infection, diarrhea, hepatitis, and neurological diseases in MHV-susceptible strains of mice (3, 53). For the A59 strain of MHV (MHV-A59), the viral attachment protein is the 180-kDa spike glycoprotein, which forms the large peplomers characteristic of the coronavirus envelope and functions as a protease-activated cell-fusing molecule (11, 13, 15, 21, 44, 46). Monoclonal antibodies (MAbs) directed against spike glycoprotein inhibit viral infectivity and/or virus-induced cell fusion (13, 54). Although some coronaviruses express a hemagglutinin-esterase glycoprotein that binds 9-O-acetylated sialic acid (30, 51), MHV-A59 does not express hemagglutinin-esterase glycoprotein (51), so its spike glycoprotein determines receptor recognition. In vitro binding studies have shown that MHV-A59 bound specifically to 110- to 120-kDa glycoproteins in BALB/c and Swiss Webster mouse liver and intestinal brush border membranes (10, 59). Expression of this receptor glycoprotein appears to play a strong role in the tissue tropism and species specificity of MHV-A59. The receptor is expressed in largest amounts on the plasma membranes of hepatocytes and brush border membranes of enterocytes from MHV-susceptible mice (10, 58). Adult SJL/J mice do not express a virus-binding protein on hepatocytes or intestine and are highly resistant to MHV infection (10, 25, 39, 43, 59). A MAb directed against the 110- to 120-kDa glycoprotein (MAb CC1) blocks virus infection of mouse fibroblasts in vitro and reduces the titer of virus in natural target tissues in vivo (38, 59). N-terminal amino acid sequencing of the 110- to 120-kDa glycoprotein immunoaffinity purified from Swiss Webster mouse liver with MAb CC1 revealed a 25-amino-acid sequence with high homology to the carcinoembryonic antigen (CEA) family of glycoproteins (58). Serological cross-reactivity was demonstrated between the 110- to 120-kDa protein from mouse liver and antibodies directed against human CEA-related proteins (58). The CEA family of glycoproteins is quite large and complex; more than 12 proteins and at least four CEA family members from humans and rodents, respectively, have been defined and partially cloned and sequenced (60). Thus, it is of critical importance to determine which of the murine CEA-related proteins can serve as MHV receptors (MHVR).

We undertook the present study to clone the cDNA for the MHVR and to study its role in determining the species specificity of MHV infection. A segment of cDNA from BALB/c mouse colon was amplified by RNA polymerase chain reaction (PCR) by using degenerate oligonucleotide primers on the basis of the N-terminal amino acid sequence of the 110- to 120-kDa MHVR glycoprotein and primers derived from a partial cDNA clone of a murine CEA-related glycoprotein (5, 58, 59). We used the resulting PCR product to isolate and characterize a full-length cDNA clone, $MHVR_1$, from BALB/c mouse liver. Transfection of the cDNA clone into hamster and human cells resulted in the synthesis of the receptor glycoprotein and rendered the cells susceptible to infection with MHV-A59. Thus, expression of the 110- to 120-kDa CEA-related mouse glycoprotein in human and hamster cells is sufficient to overcome the species barrier to MHV infection, suggesting that the absence of a functional receptor for MHV on nonmurine species determines the species specificity of MHV.

MATERIALS AND METHODS

Preparation of mouse RNA and DNA. Total cellular RNA was prepared from colon, small intestine, and liver tissues of BALB/c mice by the RNAzol B method (12). The RNA

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pellets obtained were resuspended in water, and LiCl was added to a final concentration of 3 M. After a 3-h incubation on ice, the precipitated RNA was pelleted at 4°C and washed with 70% ethanol before the final resuspension in H_2O. Poly(A)$^+$ RNA was isolated with the PolyA Tract mRNA isolation system (Promega, Madison, Wis.) according to the manufacturer's instructions. Genomic DNA was prepared from BALB/c mouse liver (9) and digested with the indicated restriction enzymes according to manufacturer's instructions (Life Technologies, Gaithersburg, Md.).

RNA and DNA PCR. Four micrograms of total RNA or 0.5 μg of poly(A)$^+$ RNA was mixed with 0.5 μg of the antisense primer. cDNA was synthesized with 500 U of Moloney murine leukemia virus reverse transcriptase RNase H$^-$ (Life Technologies) and 0.5 mM (each) all four deoxynucleotide triphosphates at 37°C for 45 min. The reaction was then terminated by heating the mixture at 95°C for 10 min. For PCR amplification of the cDNA product, the 25-μl reverse transcriptase reaction mixtures were mixed with 0.65 mM deoxynucleotide triphosphates, 0.5 μg of the same antisense primer used to prime the reverse transcriptase reaction, 1 μg of the sense primer (for degenerate oligonucleotides), or 0.5 μg of nondegenerate sense primers and 2.5 U of Amplitaq (Perkin-Elmer Cetus, Norwalk, Conn.) in a 100-μl reaction mixture overlaid with mineral oil. The products of 30 cycles (94°C for 1.5 min, 45°C for 1.5 min, and 72°C for 2 min) were analyzed by Southern blot hybridization with the appropriate ^{32}P-labeled oligonucleotide probe (34).

The oligonucleotides were synthesized with an Applied Biosystems DNA synthesizer (Applied Biosystems, Foster City, Calif.). Within the sequences of the first three primers, the nucleotides in parentheses indicate positions of degeneracy. The underlined 5′-terminal sequence of primer 5 is a *Sal*I restriction enzyme site added to aid in cloning the PCR product. The following primer sequences are shown in the 5′ to 3′ orientation: 1, GA(GA)GT(GA)AC(CA)AT(CT)GA (GA)GC(CT)GT; 2, GA(GA)GT(GA)AC(CA)AT(TC)GA (GA)GC(GA)GT; 3, CAGGTGGC(TC)GA(GA)GA(CT)AA (CT)AA(CT)G; 4, GTAGACTCCCATATCCTTCATGG; 5, GGTCGACTGGGGCTTCTCATTGATAAG; 6, CGGCAG AGAGATAATATACAG.

Northern (RNA) and Southern analyses. Total RNA was electrophoresed under denaturing conditions (48) in a 1.2% agarose gel and transferred to a Nylon membrane (Nytran; Schleicher & Schuell, Keene, N.H.). The membrane was hybridized overnight at 42°C with cDNA labeled with [^{32}P]-dCTP by nick translation. After hybridization, the filter was washed at a final stringency of 0.1× SSPE (1× SSPE is 0.18 M NaCl, 10 mM $NaPO_4$, and 1 mM EDTA [pH 7.7])–0.1% sodium dodecyl sulfate (SDS) at 60°C.

For genomic DNA analysis, 10 μg of DNA was digested with either *Eco*RI or *Sst*I. The digested DNA was electrophoresed on an 0.8% agarose gel and then transferred to Nytran (Schleicher & Schuell) according to the method of Southern (40). The membrane was prehybridized for 2 h and allowed to hybridize overnight with the ^{32}P-labeled probe. The filter was washed at a final stringency of 0.1× SSPE–0.1% SDS at 55°C.

Subcloning of PCR products and phage DNA, library screening, and DNA sequencing. The products of three amplification reactions starting from BALB/c mouse colon RNA and using oligonucleotides 3 and 4 as primers were pooled, treated with T4 DNA polymerase (Promega), purified on a Centricon 100 (Amicon, Beverly, Mass.), and ligated into pUC18 to produce the subclone designated p231. The amplification product obtained from BALB/c mouse colon RNA with primers 3 and 5 was excised and purified from a 1.5% agarose gel, treated with T4 DNA polymerase, digested with *Sal*I, and subcloned in Bluescript SK+ (Stratagene, La Jolla, Calif.). This construct of BALB/c mouse colon cDNA was named for its length, p710. cDNA clones p10.3, p10.4, and $MHVR_1$ were obtained by screening a lambda gt11 library of BALB/c mouse liver cDNA (Promega) with a nick-translated p710 insert (8). After the third round of screening, phage from positive plaques were amplified in a 20-μl PCR mixture by using the lambda gt11 forward- and reverse-sequencing primers 1218 and 1222 (New England Biolabs, Beverly, Mass.) (18). Clones p10.4 and p10.3 were constructed by subcloning the amplified inserts in the *Eco*RI-*Not*I sites of Bluescript SK+, while the insert from the isolated phage DNA was used to generate $pMHVR_1$. To obtain $pRMHVR_1$, $MHVR_1$ was excised from Bluescript by digestion with *Hin*dIII and *Not*I and ligated into the *Hin*dIII-*Eag*I sites of the expression plasmid pRSVneo. The use of these cloning sites caused the loss of the gene for neomycin resistance.

DNA sequencing of the two strands of the clones obtained was performed by the dideoxy method (35) with modified bacteriophage T7 DNA polymerase (U.S. Biochemicals, Cleveland, Ohio) or with the model 373A DNA sequencing system (Applied Biosystems).

Transient and stable expression of $MHVR_1$ in cell lines. For transient- and stable-expression studies, we used $MHVR_1$ subcloned behind the strong promoter, Rous sarcoma virus long terminal repeat ($pRMHVR_1$). Transfection of $pRMHVR_1$ into hamster (BHK) cells was performed with an electroporator (Life Technologies) by using 10 μg of plasmid and 10^7 cells. To obtain stable cell lines, pRSVneo was mixed with $pRMHVR_1$ in a 1:10 ratio for electroporation. Two G418 resistant clones, $BHK1_{MHVR1}$ and $BHK2_{MHVR1}$, were isolated after 4 weeks of selection. Approximately 1 cell of 40 was positive for expression of MHVR glycoprotein as shown by immunofluorescence with polyclonal antireceptor antibody. To select for cells expressing the receptor glycoprotein, cells were panned on antireceptor MAb CC1. For panning, bacteriological-grade petri dishes were incubated with 10 μg of MAb CC1 per ml in 0.05 M Tris Cl, pH 9.5, for 40 min at room temperature and then were washed three times with 5% fetal bovine serum in phosphate-buffered saline (PBS). Stably transfected cell lines ($BHK1_{MHVR1}$ and $BHK2_{MHVR1}$) were trypsinized, centrifuged at 400 × *g*, and washed twice with 10 ml of 5% fetal bovine serum in PBS. Three milliliters of cell suspension containing approximately 8×10^6 cells was added to MAb CC1-coated petri dishes and incubated on a flat surface at 4°C for 60 min with gentle swirling for 30 s after the first 30 min of incubation. Nonadherent cells were then pipetted from the dish, which was subsequently washed three times with 7 ml of 1% fetal bovine serum in PBS. Adherent cells were removed by gentle scraping, centrifuged for 10 min at 400 × *g*, and propagated in medium. Human RD cells transiently expressing $pRMHVR_1$ were obtained by lipofection-mediated gene transfer according to the manufacturer's recommendations (Life Technologies).

Detection of viral antigens by immunofluorescence. At 56 h after transfection of RD cells or 24 h after transfection of BHK cells, cells transiently transfected with $pRMHVR_1$ or pRSVneo were challenged with MHV-A59 (4×10^6 PFU) for 1 h. At 7.5 h postinfection, the cells on the coverslips were washed with PBS, fixed in acetone at −20°C for 8 min, air dried, and frozen at −20°C. Stably transfected BHK cells were incubated for 1 h at 37°C with MHV-A59, rat corona-

virus (RCV) (obtained from D. Percy, University of Guelph, Guelph, Ontario, Canada) (1.6×10^5 PFU), or human coronavirus (HCV)-229E and then incubated at the indicated temperatures for 7.5 h before fixation. Acetone-fixed coverslips were rehydrated for 10 min in PBS containing 2% normal goat serum or normal rabbit serum, incubated with 50 μl of a 1:50 dilution of mouse polyclonal anti-MHV-A59 convalescent serum, mouse anti-RCV serum (kindly provided by A. Smith, Yale University), or goat anti-HCV-229E serum for 1 h at 37°C. After four 15-min washes in PBS containing 2% normal goat serum or 2% normal rabbit serum, the coverslips were incubated with rhodamine-labeled goat anti-mouse immunoglobulin G (IgG) or rhodamine-labeled rabbit anti-goat IgG for 45 min at 4°C, washed, and examined with a Zeiss fluorescence microscope. Positive controls for viral infection and detection of viral antigens by immunofluorescence were L2 cells infected with MHV-A59, L2 cells susceptible to RCV (obtained from D. Percy, University of Guelph) infected with RCV, and WI38 cells infected with HCV-229E.

Preparation of polyclonal rabbit antireceptor antibody. MHVR glycoprotein, immunoaffinity purified from Swiss Webster mouse liver as described previously (59), was mixed with Freund's complete adjuvant and injected subcutaneously into female New Zealand White rabbits. Immunoaffinity-purified receptor in incomplete Freund's adjuvant was injected at weekly intervals. When incubated with L2 cells at a serum dilution of more than 1/1,200, this antiserum blocked MHV-A59 infection in vitro. The polyclonal antibody was preabsorbed against BHK cells before being used in the immunoblot and immunofluorescence analyses of the BHK_{MHVR1} cell lines.

Blocking of virus infection by monoclonal antireceptor antibody CC1. BHK cells or RD cells transiently transfected with $pRMHVR_1$ on coverslips were incubated with 5 ml of a 1:5 dilution of MAb CC1 supernatant or, as an antibody-negative control, mock treated with a 1:5 dilution of HT medium (RPMI 1640, 10% fetal bovine serum, 10% NCTC-109 medium, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM hypoxanthine, 0.016 mM thymidine, 1 mM oxalacetic acid, 100 U of insulin, 50,000 U of penicillin, 50,000 U of streptomycin, 125 μg of fungizone, 50 ng of gentamicin) at 37°C for 1 h. Antibody was removed and the cells were challenged with 5×10^7 PFU of MHV-A59 per ml, incubated at 37°C for 1 h, acetone fixed 7.5 h postinfection, and examined by immunofluorescence for the presence of viral antigens.

Membrane preparations and plaque assay. Intestinal brush border membranes were prepared as described previously (23). Liver membranes, Swiss Webster mouse liver immunoaffinity-purified MHVR protein, and crude membrane preparations of $BHK1_{MHVR1}$ and $BHK2_{MHVR1}$ cells were prepared as described previously (10, 58). MHV-A59 propagation and plaque assays were performed as described previously (45, 47).

Nucleotide sequence accession number. The sequences of cDNA clones described here have been deposited with GenBank, and accession numbers M64035 and M77196 have been assigned to clone p10.4 (MHVR in GenBank) and MHVR1, respectively.

RESULTS

RNA PCR amplification of partial receptor cDNA clones. Although amino-terminal amino acid sequencing of affinity-purified receptor, biochemical analysis, and serological cross-reactivity strongly suggested that the MHVR glycoprotein was a murine member of the CEA family of glycoproteins (58), no full-length cDNA clone of murine CEA-related proteins was available for expression studies. To obtain partial MHVR cDNA clones, we used RNA PCR to amplify cDNAs derived from BALB/c mouse colon, the tissue which expresses the highest level of receptor protein (58). A set of primers based on the first 17 N-terminal amino acids of the affinity-purified receptor (sense primers) and on a partial cDNA sequence of a murine CEA family member (antisense primers) was designed (5). Two 64-fold degenerate oligonucleotide pools (primers 1 and 2) were designed from the first seven amino acids, and a 32-fold degenerate oligonucleotide pool (primer 3) based on amino acids 10 to 17 of the mature receptor protein was designed. A rodent codon usage table was consulted when designing the oligonucleotide primers to avoid a higher degree of degeneracy. Three oligonucleotide primers from the published murine CEA sequence were selected by computer program (29). Two of them (primers 4 and 5) were used as antisense primers, and the third (internal) one (primer 6) was used as a probe for detection of a CEA-related product. Total RNA of BALB/c mouse colon was reverse transcribed with oligonucleotide 4 to prime the reaction. The resulting cDNA was amplified in separate PCRs with sense primers 1, 2, or 3 paired with primer 4. On the basis of the alignment of the partial murine CEA-related cDNA clone (5) and the human CEA glycoprotein (4), and assuming that the two proteins share an amino terminus, we predicted the sizes of the PCR amplification products. The product generated with sense primer 1 or 2 and antisense primer 4 would be 258 bp, and the product generated with sense primer 3 and the antisense primer 4 would be 231 bp. Figure 1 shows that the PCR products had the expected sizes and hybridized with the internal oligonucleotide. The doublet detected by hybridization is due to depletion of the degenerate oligonucleotides in the PCR, resulting in the production of both single- and double-stranded products which are resolved on the gel. Amplification of cDNAs derived from BALB/c mouse small intestine

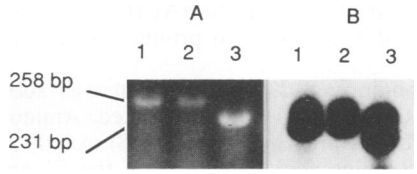


FIG. 1. Amplification of cDNA with oligonucleotide primers derived from the N-terminal amino acid sequence of the MHVR and from a region within the sequence of a partial murine CEA-related glycoprotein cDNA. Total BALB/c mouse colon RNA was reverse transcribed with antisense primer 4, selected from the cDNA sequence in reference 5. The cDNA was then amplified with degenerate sense oligonucleotides based on amino acids 1 to 7 (primer 1, lanes 1; primer 2, lanes 2) or 10 to 17 (primer 3, lanes 3) of the partial N-terminal amino acid sequence of immunoaffinity-purified MHVR glycoprotein (58, 59) paired with antisense primer 4. PCRs of 30 cycles of 94°C for 1.5 min, 45°C for 1.5 min, and 72°C for 2 min were performed, and results were analyzed by agarose gel electrophoresis (A) and Southern blot hybridization (B) (40) by using ^{32}P-labeled primer 6. Size markers confirm 258-bp amplification products for lanes 1 and 2 and a 231-bp product for lanes 3. The doublets seen on the Southern blot result from the presence of both double-stranded DNA and the single-stranded DNA product that accumulates following the exhaustion of the degenerate primers in the PCR.

RNA and poly(A)$^+$ RNA from BALB/c mouse liver by using primers 3 and 4 also yielded products of 231 bp (data not shown).

The colon-derived 231-bp fragment was subcloned into Bluescript (named p231) and sequenced. Amino acids 17 to 25 of the MHVR protein sequence (58) exactly matched the predicted 8-amino-acid sequence at the 5′ end of p231. Comparison of the MHVR PCR product to the published partial cDNA clone of mouse CEA-related protein revealed two nucleotide differences corresponding to the first two amino acids of the published partial cDNA clone (5).

To obtain a larger clone for library screening, BALB/c mouse colon RNA was amplified with sense primer 3 and antisense primer 5. The nucleotide sequence of the PCR product (p710) was identical at its 5′ end to that of p231 and continued in the same open reading frame to include the nucleotide sequence of the partial cDNA clone of the murine CEA-related protein (5) except for nucleotides 307 and 311. Thus, by RNA PCR we identified and cloned a cDNA from BALB/c mouse colon which had 5′ amino acid sequence identity to the MHVR glycoprotein and strong 3′ nucleotide sequence homology to mmCGM1 (*Mus musculus* carcinoembryonic antigen gene family member), a CEA-related glycoprotein of mice.

Isolation of cDNA clones encoding a protein with amino acid sequence of the MHVR. To isolate full-length cDNA clones encoding MHVR, a cDNA library from BALB/c mouse liver was screened with p710. Three cDNA clones were obtained after screening 500,000 plaques: MHVR10.4, MHVR10.3, and $MHVR_1$, which had insert sizes of 1.4, 1.1, and 2.6 kb, respectively, and ended with poly(A) tails. The inserts were then subcloned into the *Eco*RI and *Not*I sites of Bluescript, yielding clones p10.3, p10.4, and $pMHVR_1$ (Fig. 2A). The largest clone isolated, $MHVR_1$, contained 69 bases of 5′ untranslated sequence, 102 bases coding for the 34-amino-acid leader peptide, 1,272 nucleotides coding for the mature protein, and 1,189 nucleotides of 3′ untranslated sequence to the poly(A) tail. The partial cDNA clones p10.4 and p10.3 started at bases 197 and 547 of $MHVR_1$, respectively, and their sequences were identical up to base 1,586 of $MHVR_1$, where their poly(A) tails started. Figure 2B shows the nucleotide and predicted amino acid sequences of the MHVR. The first 25 amino acids of the mature protein predicted by the nucleic acid sequence were identical to the amino acids determined by N-terminal amino acid sequencing of the affinity-purified MHVR (58).

Expression of the $MHVR_1$ cDNA clone. To determine whether the cDNA clone $MHVR_1$ encodes a functional MHVR, we subcloned it into the expression vector pRSV-neo to yield $pRMHVR_1$. We transiently expressed $pRMHVR_1$ in two cell lines that normally are completely resistant to MHV infection, the BHK line of hamster fibroblasts (16) and the RD line of human rhabdomyosarcoma cells. Figure 3 shows that MHV-A59 infected hamster or human cell lines only after these had been transfected with $pRMHVR_1$ (Fig. 3A and E, respectively) and that MHV-A59 did not infect cells transfected with pRSVneo alone (Fig. 3B and F). To determine whether the MHVR glycoprotein was the only portal of entry of the virus into these cells, we pretreated transfected cells with the antireceptor MAb CC1 or an irrelevant MAb of the same isotype and then challenged them with infectious MHV-A59. Figure 3C and G show that no viral antigens were detected in the cytoplasm of virus-challenged cells expressing $pRMHVR_1$ when the receptor glycoprotein was blocked with MAb CC1. No inhibition of virus infection was observed when the irrelevant MAb was used (data not shown). Thus, MAb CC1 prevented MHV-A59 infection of BHK cells and RD cells transfected with the $MHVR_1$ cDNA.

BHK cell lines stably transfected with $pRMHVR_1$ ($BHK1_{MHVR1}$, $BHK2_{MHVR1}$) were challenged with MHV-A59. Development of viral antigens and virus-induced cell fusion were observed with both of the $pRMHVR_1$-transfected BHK cell lines 7 h after inoculation with MHV-A59 (Fig. 3D shows results for $BHK2_{MHVR1}$). MHV-induced cell fusion was prevented by pretreatment of the transfected cell lines with MAb CC1.

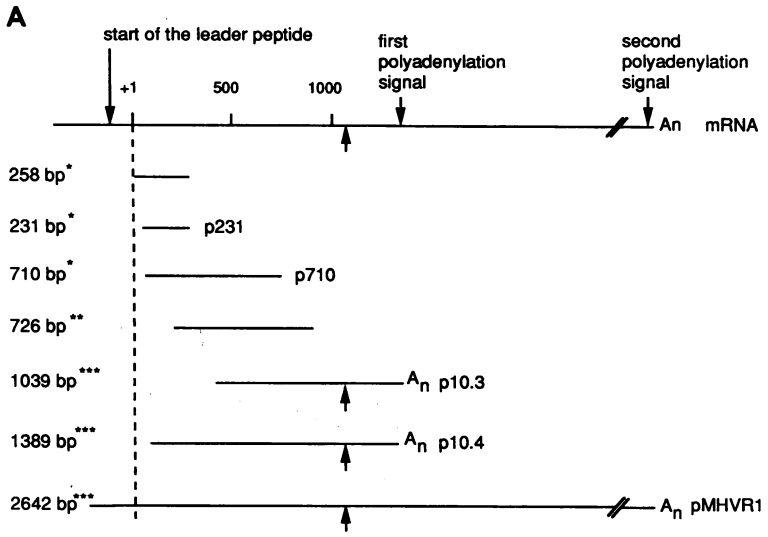
Expression of MHVR glycoprotein on the membranes of BHK cell lines stably transfected with $pRMHVR_1$ was analyzed by immunoblotting with polyclonal antireceptor rabbit antibody preabsorbed with BHK cells. Figure 4A shows the specificity of the polyclonal antireceptor antibody for the 110- to 120-kDa receptor in BALB/c mouse intestine and liver and immunoaffinity-purified receptor from Swiss Webster mouse liver. Figure 4B shows that the $BHK1_{MHVR1}$ and $BHK2_{MHVR1}$ cell lines expressed a 120-kDa glycoprotein which was recognized by the polyclonal antireceptor antibody. This glycoprotein was also recognized by MAb CC1 (data not shown). The size of the receptor glycoprotein expressed in the stable cell lines corresponded to the size of the MHVR in BALB/c mouse intestinal brush borders.

The growth of MHV-A59 in $BHK2_{MHVR1}$ cells was assessed to determine whether the presence of the receptor could permit productive virus infection. As shown in Table 1, $BHK2_{MHVR1}$ produced MHV-A59 at titers comparable to those from mouse L2 cells.

The virus specificity of the cloned MHVR was investigated by challenging $BHK2_{MHVR1}$ cells with RCV and HCV-229E. At 8 h postinoculation, the cells were fixed in acetone and the presence of viral antigens was determined by immunofluorescence with the appropriate antiviral antibodies. Neither RCV nor HCV-229E was able to infect $BHK2_{MHVR1}$ cells and produce viral proteins (data not shown). Thus, the receptor encoded by $MHVR_1$ is specific for mouse coronavirus.

Northern and Southern blot analyses of the MHVR. To determine the size of the mRNA transcript that encodes the MHVR glycoprotein, Northern blot hybridization was performed with total RNA prepared from BALB/c mouse colon and small intestine. The hybridization probe employed was the *Eco*RI-*Not*I-liberated insert of p10.4. After washing under stringent conditions, a 3.4-kb transcript was detected in both tissues on an overnight exposure (Fig. 5A). When the blot was exposed to film for a week, a second major transcript of 1.7 kb was detected in both tissues. Additional minor bands which appeared during this longer exposure probably represent transcripts of more distantly related mmCGMs (6). Figure 5B shows a Southern blot of BALB/c mouse liver DNA digested with *Eco*RI or *Sst*I and probed with the p10.4 insert. A wide range of band intensities was observed on the autoradiogram. The darkest bands represent the hybridization of the most homologous gene(s), the bands of intermediate intensity may represent related genes, and the lightest bands may arise from cross-hybridization with distantly related genes.

The MHVR is a member of the Ig superfamily. More than 10 cross-reactive human isoantigens in the CEA family have been detected in a variety of normal and tumor tissues (37). Proteins in this family have several domains in common: a 34-amino-acid leader peptide, an N-terminal domain of 108 to 110 amino acids that includes an Ig-like variable region, two to six Ig constant (C2) region-like domains defined by



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                                                                                M  E  L  A  S  A  H  L  H  K  G    -24
1    GGCAGATATCAGGGCAGCCTGGCTTAGCAGTAGTGTTGGAGAAGAAGCTAGCAGGCAGGCAGCAGAGACATGGAGCTGGCCTCAGCACATCTCCACAAAG

       Q  V  P  W  G  G  L  L  L  T  A  S  L  L  A  S  W  S  P  A  T  T  A [E] V  T  I  E  A  V  P  P  Q     10
101  GGCAGGTTCCCTGGGGAGGACTACTGCTCACAGCCTCACTTTTAGCCTCCTGGAGCCCTGCCACCACTGCTGAAGTCACCATTGAGGCTGTGCCGCCCCA

       V  A  E  D  N  N  V  L  L  L  V  H  N  L  P  L  A  L  G  A  F  A  W  Y  K  G  N  T  T  A  I  D  K     43
201  GGTTGCTGAAGACAACAATGTTCTTCTACTTGTTCACAATCTGCCCCTGGCGCTTGGAGCCTTTGCCTGGTACAAGGGAAACACTACGGCTATAGACAAA

       E  I  A  R  F  V  P  N  S  N  M  N  F  T  G  Q  A  Y  S  G  R  E  I  I  Y  S  N  G  S  L  L  F  Q  M     77
301  GAAATTGCACGATTTGTACCAAATAGTAATATGAATTTCACGGGGCAAGCATACAGCGGCAGAGAGATAATATACAGCAATGGATCCCTGCTCTTCCAAA

       I  T  M  K  D  M  G  V  Y  T  L  D  M  T  D  E  N  Y  R  R  T  Q  A  T  V  R  F  H  V  H  P  I  L    110
401  TGATCACCATGAAGGATATGGGAGTCTACACACTAGATATGACAGATGAAAACTATCGTCGTACTCAGGCGACTGTGCGATTTCATGTACACCCCATATT

       L  K  P  N  I  T  S  N  N  S  N  P  V  E  G  D  D  S  V  S  L  T  C  D  S  Y  T  D  P  D  N  I  N    143
501  ATTAAAGCCCAACATCACAAGCAACAACTCCAATCCCGTGGAGGGTGACGACTCCGTATCATTAACCTGTGACTCTTACACTGACCCTGATAATATAAAC

       Y  L  W  S  R  N  G  E  S  L  S  E  G  D  R  L  K  L  S  E  G  N  R  T  L  T  L  L  N  V  T  R  N  D    177
601  TACCTGTGGAGCAGAAATGGTGAAAGCCTTTCAGAAGGTGACAGGCTGAAGCTGTCTGAGGGCAACAGGACTCTCACTTTACTCAATGTCACGAGGAATG

       T  G  P  Y  V  C  E  T  R  N  P  V  S  V  N  R  S  D  P  F  S  L  N  I  I  Y  G  P  D  T  P  I  I    210
701  ACACAGGACCCTATGTGTGTGAAACCCGGAATCCAGTGAGTGTCAACCGAAGTGACCCATTCAGCCTGAACATTATCTATGGTCCGGACACCCCGATTAT

       S  P  S  D  I  Y  L  H  P  G  S  N  L  N  L  S  C  H  A  A  S  N  P  P  A  Q  Y  F  W  L  I  N  E    243
801  ATCCCCCTCAGATATTTATTTGCATCCAGGGTCAAACCTCAACCTCTCCTGCCATGCAGCCTCTAACCCACCTGCACAGTACTTTTGGCTTATCAATGAG

       K  P  H  A  S  S  Q  E  L  F  I  P  N  I  T  T  N  N  S  G  T  Y  T  C  F  V  N  N  S  V  T  G  L  S    277
901  AAGCCCCATGCATCCTCCCAAGAGCTCTTTATCCCCAACATCACTACTAATAATAGCGGAACCTATACCTGCTTCGTCAATAACTCTGTCACTGGCCTCA

       R  T  T  V  K  N  I  T  V  L  E  P  V  T  Q  P  F  L  Q  V  T  N  T  T  V  K  E  L  D  S  V  T  L    310
1001 GTAGGACCACAGTCAAGAACATTACAGTCCTTGAGCCAGTGACTCAGCCCTTCCTCCAAGTCACCAACACCACAGTCAAAGAACTAGACTCTGTGACCCT

       T  C  L  S  N  D  I  G  A  N  I  Q  W  L  F  N  S  Q  S  L  Q  L  T  E  R  M  T  L  S  Q  N  N  S    343
1101 GACCTGCTTGTCGAATGACATTGGAGCCAACATCCAGTGGCTCTTCAATAGCCAGAGTCTTCAGCTCACAGAGAGAATGACACTCTCCCAGAACAACAGC

       I  L  R  I  D  P  I  K  R  E  D  A  G  E  Y  Q  C  E  I  S  N  P  V  S  V  R  R  S  N  S  I  K  L  D    377
1201 ATCCTCAGAATAGACCCTATTAAGAGGGAAGATGCCGGCGAGTATCAGTGTGAAATCTCGAATCCAGTCAGCGTCAGGAGGAGCAACTCAATCAAGCTGG

       I  I  F  D  P  T  Q  G  G  L  S  D  G  A  I  A  G  I  V  I  G  V  V  A  G  V  A  L  I  A  G  L  A    410
1301 ACATAATATTTGACCCAACACAAGGAGGCCTCTCAGATGGCGCCATTGCTGGCATCGTGATTGGAGTTGTGGCTGGGGTGGCTCTAATAGCAGGGCTGGC

       Y  F  L  Y  S  R  K  S  G  G  S  G  S  F  *                                                          424
1401 ATATTTCCTCTATTCCAGGAAGTCTGGCGGATCTGGCTCCTTCTGACAACTCTCCTAACAAGGTGGATGACGTCGCATACACTGTCCTGAACTTCAATTC

1501 CCAGCAACCCAACCGGCCAACTTCAGCCCCTTCTTCTCCAAGAGCCACAGAAACAGTTTATTCAGAAGTAAAAAAGAAGTGAGCATAATCTGTCCGTCTG
1601 TCCTGCTGGCTGCACCAGTGATGCATTCCCGGATTCTGTTCCTCACTGGAGGGTCTCAGCACACACACACACGTACACATGCGCGCGCGCACACACACAC
1701 ACACACACACACACACACACTTACACACACACTCATGCATTCACTCTATTGACTCCTTCAGTGTCTATAGAAGAAAAGGTGGATCCTGGAGCCTACAGAA
1801 AACTCAACCCTTCTAGGCTTTCAAATTTGGCTGAGAGTGAGGTATCAAAATTTCTCACCCTTTCACTTTCCTGACCCAGATTGTTGAAAATTGACCTATT
1901 CAGAGCACCTTCATTCCCCTCCCAACTCCAAGTCCTGCCCTATCAGAGTCTGACTTGAATTTCCATAAACCTTGGAGGTCACCTAAGTGCTTACGCCAAA
2001 CAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAAACAAACCAGAAGCAGGAAATGGCCAGTCCCATATCTTTAAAGGCTGATTGGAAGCC
2101 ACCATACATGAGAAGATCAAACCTCCATGGGCAATCTACACACCCGACAACTGTCATGCTTACCCATCTGGGACATTCGAGTCTCTGAACCTTGTGCCCT
2201 CACGCCTGAGCCCTTCTCTGAGCCTTTCTCCAGAAAATCCACTCACAGCAACTAGAGAGGCTCTTTGTCAGCAACTCCAAGCAAACTGCTAGGCAGGATT
2301 CAGAAGAAAAGACAGCATCTCTAACATCCACCAGGAAGGTGCCCAGAAAAGCAGAGCTGGTGACTTTGGACTGACAGACATCTGGAGTGTGAAAAAGCAG
2401 CACAGAGCTAACCTTCGGAGAGTGTTGAAATTATTTGAAAAGAAGCCATATTTGGAGGTATTGGAGTTTTCCTCTTTCTGAGACAATCCACTATTTGAAA
2501 ATTGTAGCTACTGAATTGCCTCTCAGTATGCGAGCTGATCACGTTTCCTTAGGGCCACTAGATTTCTGTCTCCCTTAGCCCCTCAAGCCCTTTTGATCAT
2601 GAGTTCCAAACCAAAAATAAATAAATGAACAGTGAGGCAGTCAAAAAAAAAAAAAAAAAAAAAA
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FIG. 2. Nucleic acid and predicted amino acid sequences for $MHVR_1$. (A) Positions of the partial clones obtained. PCR products from BALB/c mouse colon mRNA are indicated by *, the partial murine CEA-related cDNA sequence from reference 5 is indicated by **, and cDNA clones isolated from the BALB/c mouse liver cDNA library are indicated by ***. Names of any cloned products are given. The arrow indicates the position of the stop codon. (B) Nucleic acid sequence of $MHVR_1$ (numbered on left) and its deduced amino acid sequence (single-letter code) (numbered on right). The first amino acid of the mature protein is enclosed in brackets. The 16 potential N-linked glycosylation sites are underlined, the stop codon is indicated by *, and the potential polyadenylation signal at the end of the clone is double underlined. The polyadenylation signal for clones p10.3 and p10.4 begins at nucleotide 1566.

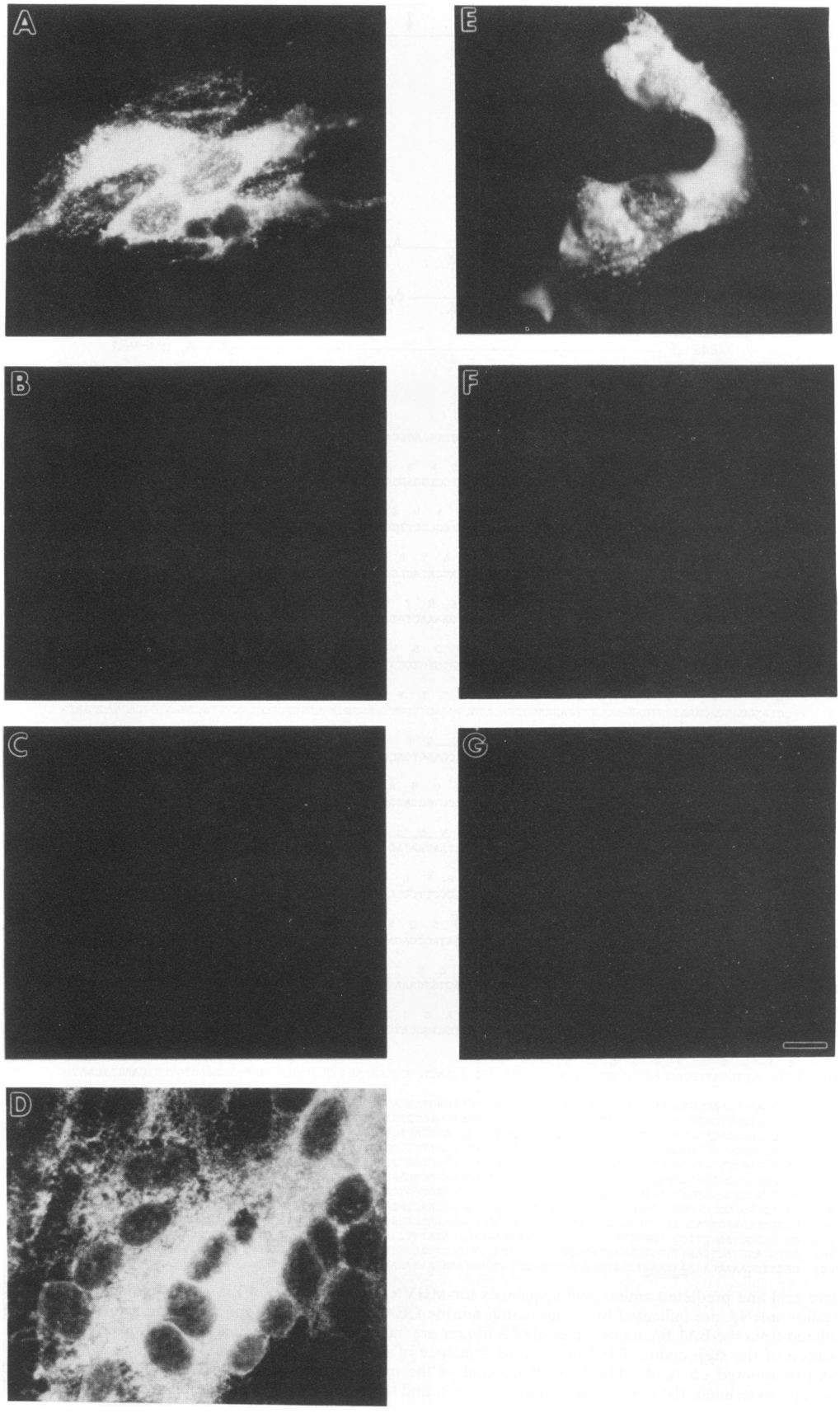
A
E
B
F
C
G
D

FIG. 3. Immunofluorescence of MHV-A59 antigens in MHV-infected hamster and human cells transfected with the $MHVR_1$ cDNA clone. BHK cells (A to D) and RD cells (E to G) were transiently (A to F) or stably (G) transfected with either $pRMHV_1$ (A, C, D, E, and G) or pRSVneo alone (B and F). Cells in panels C and G were pretreated with antireceptor MAb CC1, and then all cultures were challenged with MHV-A59. Viral antigens were detected 7.5 h after virus inoculation by using mouse anti-MHV serum and rhodamine-labeled goat anti-mouse IgG. Magnification, ×315.

disulfide bonds, and either a transmembrane domain and short C-terminal domains (57) or a phosphotidylinositol glycolipid-linked membrane attachment without transmembrane or cytoplasmic domains. The structure of the MHVR glycoprotein deduced from the sequence of $MHVR_1$ is shown in Fig. 6. This proposed structure is based on homologies with other members of the Ig superfamily. Its putative extracellular region could form an initial loop similar to an Ig-variable domain stabilized by a salt bridge (57). This is followed by three disulfide-linked loops of 40 to 49 amino acids which resemble the constant (C2) domains of immunoglobulins. Near the carboxyl terminus, a potential transmembrane domain was identified by hydrophilicity analysis according to both the Kyte-Doolittle (26a) and the Hopp-Woods (21a) methods, as shown in Fig. 7. Comparison of the amino acid sequence and domain structure of the MHVR with those of CEA-related glycoproteins showed that MHVR was most homologous to human biliary glycoprotein I (19) and rat liver ecto-ATPase (28).

DISCUSSION

This study demonstrates that expression of a cDNA clone of a murine glycoprotein in the CEA family of glycoproteins is sufficient to permit infection of human or hamster cells with murine coronavirus. This extends our previous studies which identified the MHVR as a murine member of the CEA family of glycoproteins in the Ig superfamily. N-terminal amino acid sequencing of immunoaffinity-purified MHVR revealed strong homology with human and rat glycoproteins in the CEA family (26, 58, 59, 60), and antigenic cross-reactivity of the immunoaffinity-purified MHVR with antiserum to human CEA was demonstrated previously (58).

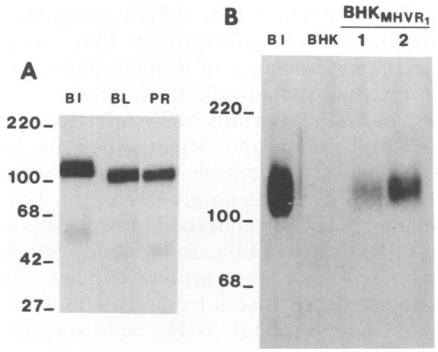

FIG. 4. Immunoblot of MHVR glycoprotein in mouse tissues and in hamster cells transfected with cDNA clone $MHVR_1$, with rabbit polyclonal antibody against immunoaffinity-purified receptor. For the immunoblot shown in panel A, 50 μg of BALB/c mouse intestinal brush border membrane proteins (BI) and BALB/c mouse liver membrane proteins (BL) and less than 100 ng of MHVR glycoprotein immunoaffinity purified from Swiss Webster mouse liver (PR) were electrophoresed, blotted, and incubated with a 1:500 dilution of rabbit polyclonal antiserum against immunoaffinity-purified MHVR. For the immunoblot shown in panel B, 1 μg of BI, 100 μg of membrane proteins prepared from nontransfected BHK cells (BHK), and 100 μg of membrane proteins prepared from the $BHK1_{MHVR1}$ and $BHK2_{MHVR1}$ cell lines were electrophoresed, blotted, and incubated with a 1:500 dilution of the rabbit polyclonal antiserum against immunoaffinity-purified MHVR preabsorbed with BHK cells. The blots were then incubated with ^{125}I-protein A, washed, and exposed to film. Positions of molecular size standards (in kilodaltons) are shown to the left of each immunoblot.

Isolation and characterization of clones of MHVR. Although partial sequences of murine CEA-related glycoprotein had been published previously (5), no nucleotide sequence data for the amino termini of the murine CEA-related proteins were available. We therefore used PCR to demonstrate that our N-terminal amino acid sequence and the partial murine CEA-related cDNA clone were part of the same molecule and to prepare a probe with which to obtain cDNA clones. To date, partial cDNA clones for four different murine CEA-related transcripts have been identified (5, 6, 50), but the total number of murine CEA-related genes and the splicing patterns of their transcripts have not yet been determined. The cDNA clone reported here is highly homologous to the partial clone of murine CEA-related glycoprotein, $mmCGM_1$ (5). The nucleotide sequences are nearly identical, and our clone extends the sequence 306 bases and 1,612 bases in the 5′ and 3′ directions, respectively. The differences between these two clones result in nonconservative amino acid changes at positions 46 and 47 of the MHVR glycoprotein. The MHVR sequence and the partial murine CEA-related cDNA clone $mmCGM_1$ may represent allelic forms of a single gene. The best characterized of the other three mmCGM transcripts, $mmCGM_2$ (50), encodes a glycoprotein of 42 kDa, significantly smaller than the 110- to 120-kDa protein which binds MHV (10).

Southern blot analysis of BALB/c mouse DNA with p10.4 probe identified 10 bands with a considerable range of intensity in hybridization signals. We speculate that the weaker signals are due to cross-hybridization of the probe with murine genes encoding other members of the CEA gene family. Northern blot analysis of total RNA from BALB/c mouse small intestine and colon showed an abundant 3.4-kb transcript and a much less abundant 1.7-kb mRNA which hybridized with p10.4. Similar results were reported by Beauchemin and coworkers (5) when colon and liver RNAs from mouse strain CD-1 were hybridized to the partial cDNA of $mmCGM_1$. These two transcripts could arise by alternative splicing of exons from a single gene, as shown for

TABLE 1. Virus production by L2 cells and a BHK cell line stably transfected with $pRMHVR_1$[a]

Cell line	Virus yield (log_{10} units) at time postinfection			
	4 h	10 h	18 h	24 h
L2	<2	7.0	7.3	7.4
$BHK2_{MHVR1}$	<2	5.1	7.2	7.5

[a] The two cell lines were infected with MHV-A59 at a multiplicity of infection of 2.5 for 1 h at 37°C. The monolayers were then washed and refed. Aliquots of media were removed at the times indicated, and virus production was assessed by plaque assay (see Materials and Methods). Values given are virus yield (log_{10} units) in culture medium at various times postinfection.

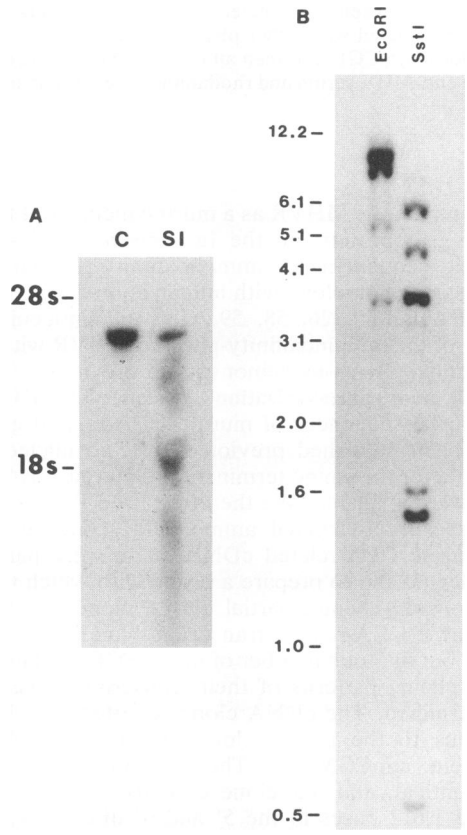

FIG. 5. Northern and Southern blot analyses of MHVR. (A) Total RNA from BALB/c mouse colon (C) and small intestine (SI) were probed with the insert of p10.4. Lane C shows a 1-day exposure, and lane SI shows a 7-day exposure. Sizes of the transcripts were calculated in relationship to the 28S and 18S ribosomal bands. (B) BALB/c mouse liver DNA (10 μg) digested with *Eco*RI or *Sst*I was electrophoresed on an 0.8% agarose gel, blotted, and hybridized to the insert of p10.4.

human biliary glycoprotein I (2, 19). Alternatively, and in our view more likely, the two mRNAs encoding the MHVR arise from inefficient termination of transcription at the end of the shorter transcript. The polyadenylation signal present in p10.3 and p10.4 is not the consensus sequence AATAAA but the less common sequence AGTAAA, which is much less effective in terminating transcription and could result in read-through transcripts (56).

The expression of the $MHVR_1$ transcript and the MHVR glycoprotein in different murine tissues correlates well with the tissue tropism of MHV infection. Williams and coworkers (58) showed by binding of radiolabeled monoclonal antireceptor antibody that the MHVR glycoprotein is present at highest levels in BALB/c mouse colon, small intestine, and liver, which are major target tissues for MHV infection. The receptor glycoprotein was not detectable in other murine tissues by this method. In this study, we showed that the 3.4-kb transcript of the MHVR gene is at least 10-fold more abundant in colon than in small intestine, while the 1.7-kb transcript is only two- to fourfold more abundant in colon than in small intestine. Tissue-specific factors which regulate the expression of the MHVR mRNA and glycoprotein could account in part for differences in susceptibility to MHV infection of mice of different ages and strains.

Relationship of the MHVR to receptors for other animal viruses. In addition to the MHVR, three members of the Ig superfamily of cell surface glycoproteins act as virus receptors, and two of these are intercellular adhesion molecules. Intercellular adhesion molecule 1 is the receptor for the major group of rhinoviruses (17, 42, 49) and binds to lymphocyte function-associated molecule 1 mediating leukocyte adhesion (41). CD4, the glycoprotein receptor for human immunodeficiency virus (14, 24, 31), is expressed on membranes of T lymphocytes and other cells susceptible to human immunodeficiency virus infection and binds to class II major histocompatibility complex molecules, serving as a lymphocyte adhesion molecule (36). The normal cellular function of the Ig superfamily member which serves as the poliovirus receptor glycoprotein is not yet known (32). The exploitation by viruses of cell membrane molecules with natural ligand binding functions as targets for virus attachment and entry is of considerable interest (27, 55). Often viruses attach to domains of the receptors different from those utilized by the natural ligand (33, 52). The cellular function(s) of the MHVR glycoprotein is not yet known. Possibly it serves the host as an adhesion molecule as suggested for another murine CEA family member, $mmCGM_2$ (50). Human CEA, which antigenically cross-reacts with the MHVR glycoprotein (58), appears to be important in the maintenance of tissue architecture and may serve as an intercellular adhesion protein (7).

Expression of MHVR permits MHV infection of nonmurine species. Cells of nonmurine origin, such as human and hamster cells, are not susceptible to infection with MHV but can be infected with viral genomic RNA (16). Expression of the cDNA clone $MHVR_1$ rendered hamster cells (BHK) and human cells (RD) susceptible to infection by MHV-A59. These transfected cells were protected from infection and from virus-induced cell fusion by pretreatment with antireceptor MAb CC1. Although BHK cells transiently transfected with $pRMHVR_1$ did not express enough protein to be detected by immunoblot analysis, BHK cell lines stably transfected with $pRMHVR_1$ produced enough of the MHVR glycoprotein to permit detection by polyclonal antireceptor

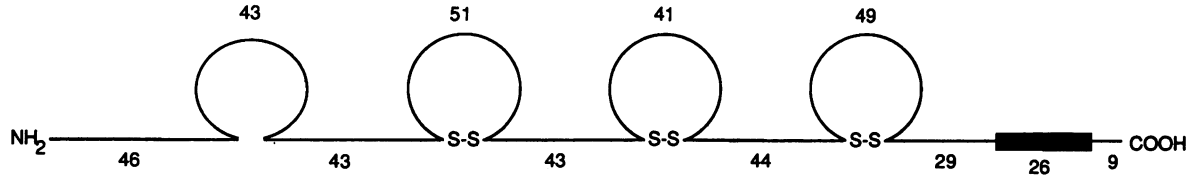

FIG. 6. Proposed domain structure for the MHVR glycoprotein. The proposed domain structure for the MHVR is based on the deduced amino acid sequence. As for other CEA-related glycoproteins, the first loop may resemble an Ig-variable domain and may be stabilized by a salt bridge. The three loops defined by disulfide bonds, which resemble C2 Ig constant domains (57), are shown, and the number of amino acids within and between each loop is indicated. The transmembrane domain is designated by a bar.

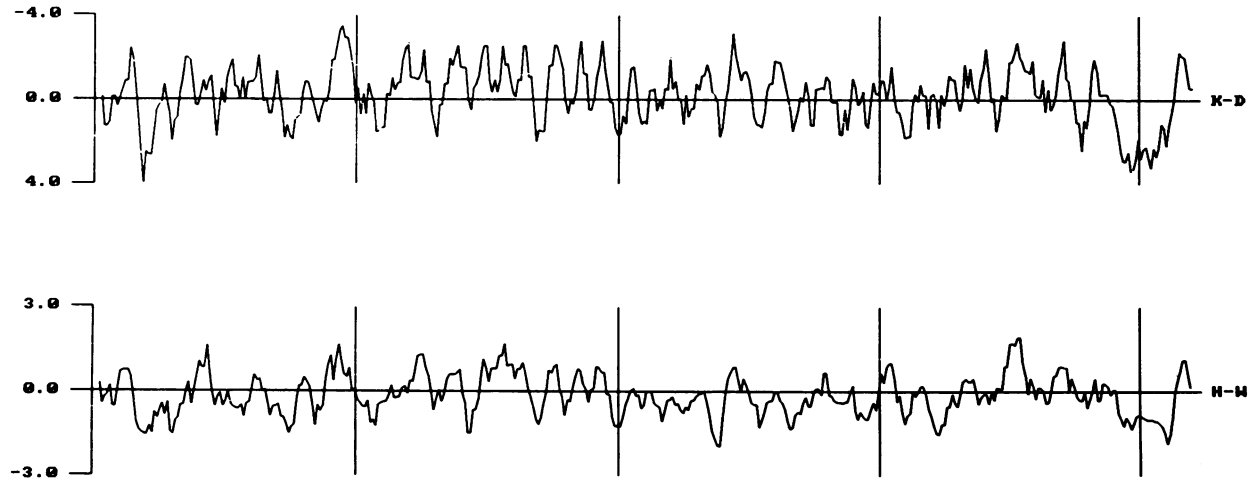

FIG. 7. Hydrophobicity analysis of the MHVR polypeptide. Hydrophobicity analysis (according to the Kyte-Doolittle [K-D] and Hopp-Woods [H-W] methods) of the MHVR, starting at amino acid 1 of the mature protein. Values above the *x* axis are hydrophilic, and those below are hydrophobic. Each subdivision represents 100 amino acids.

antibody and MAb CC1. Expression of $MHVR_1$ in the stably transfected BHK cells permitted the complete infectious cycle of MHV-A59, including release of infectious virus (Table 1). These results show that BHK cells are normally resistant to infection with MHV-A59 because they do not express a receptor for the viral spike glycoprotein. Expression of $MHVR_1$ is sufficient to permit infection of previously receptor-deficient cells. The expression of $MHVR_1$ in cells of nonmurine origin will facilitate studies of genetic recombination between MHV and other coronaviruses now restricted to cells of their normal host species.

Although the $MHVR_1$ cDNA clone has a high degree of homology with rat and human members of the CEA family of glycoproteins, RCV and HCV failed to infect the BHK cell lines expressing $pRMHVR_1$. A simple explanation of this result is that MHV spike glycoprotein binds to a mouse-specific portion of the MHVR which is not recognized by coronaviruses of other species. Thus, this experiment supports the hypothesis that the species specificity of coronaviruses in cell culture is due to the selectivity of the virus receptor.

The diversity of expression of CEA-related proteins in human tissues results from gene duplication, alternative splicing patterns, and developmentally regulated, tissue-specific control of protein expression (2). In mice, partial cDNA clones for four different CEA-related glycoproteins have been identified to date (5, 6, 50). If there is gene duplication and alternative splicing of murine CEA-related genes, then it is feasible that the domain of MHVR which binds MHV and MAb CC1 may be present on more than one murine glycoprotein. The existence of multiple related virus-binding proteins sharing one virus-binding domain has not been recognized previously on virus receptors in the Ig superfamily. It is an intriguing possibility that one of these proteins might bind virus but fail to act as a functional receptor for initiating virus infection. The intricate developmental and tissue-specific regulation of expression of CEA-related proteins (22), one or more of which can serve as an MHVR, may help to explain the dependence of mouse coronavirus infection and virulence on the age and strain of the host (1, 20, 25).

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REFERENCES

1. **Bang, F. B., and A. Warwick.** 1960. Mouse macrophages as host cells for the mouse hepatitis virus and the genetic basis of their susceptibility. Proc. Natl. Acad. Sci. USA **46**:1065–1075.
2. **Barnett, T. R., A. Kretschmer, D. A. Austen, S. J. Goebel, J. T. Hart, J. J. Elting, and M. E. Kamarck.** 1989. Carcinoembryonic antigens: alternative splicing accounts for the multiple mRNAs that code for novel members of the carcinoembryonic antigen family. J. Cell Biol. **108**:267–276.
3. **Barthold, S. W.** 1986. Mouse hepatitis virus biology and epizootiology, p. 571–601. *In* P. N. Bhatt, R. O. Jacoby, H. C. Morse III, and A. E. New (ed.), Viral and mycoplasmal infections of laboratory rodents. Effects on biomedical research. Academic Press, Inc., Orlando, Fla.
4. **Beauchemin, N., S. Benchimol, D. Cournoyer, A. Fuks, and C. P. Stanners.** 1987. Isolation and characterization of full-length functional cDNA clones for human carcinoembryonic antigen. Mol. Cell. Biol. **7**:3221–3230.
5. **Beauchemin, N., C. Turbide, D. Afar, J. Bell, M. Raymond, C. P. Stanners, and A. Fuks.** 1989. A mouse analogue of the human carcinoembryonic antigen. Cancer Res. **49**:2017–2021.
6. **Beauchemin, N., C. Turbide, J. Q. Huang, S. Benchimol, S. Jothy, K. Shirota, A. Fuks, and C. P. Stanners.** 1989. Studies on the function of carcinoembryonic antigen, p. 49–64. *In* A. Yachi and J. E. Shively (ed.), The carcinoembryonic antigen gene family. Elsevier Science Publishers BV, New York.
7. **Benchimol, S., A. Fuks, S. Jothy, N. Beauchemin, K. Shirota, and C. P. Stanners.** 1989. Carcinoembryonic antigen, a human tumor marker, functions as an intercellular adhesion molecule. Cell **57**:327–334.
8. **Benton, W. D., and R. W. Davis.** 1977. Screening lambda gt recombinant clones by hybridization to single plaques in situ. Science **196**:180–182.
9. **Blin, N., and D. W. Stafford.** 1976. A general method for isolation of high molecular weight DNA from eukaryotes.

Nucleic Acids Res. 3:2303–2308.
10. **Boyle, J. F., D. G. Weismiller, and K. V. Holmes.** 1987. Genetic resistance to mouse hepatitis virus correlates with absence of virus-binding activity on target tissues. J. Virol. **61**:185–189.
11. **Cavanagh, D., D. A. Brian, L. Enjuanes, K. V. Holmes, M. M. Lai, H. Laude, S. G. Siddell, W. Spaan, F. Taguchi, and P. J. Talbot.** 1990. Recommendations of the Coronavirus Study Group for the nomenclature of the structural proteins, mRNAs, and genes of coronaviruses. Virology **176**:306–307.
12. **Chomczynski, P., and N. Sacchi.** 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. **162**:156–159.
13. **Collins, A. R., R. L. Knobler, H. Powell, and M. J. Buchmeier.** 1982. Monoclonal antibodies to murine hepatitis virus-4 (strain JHM) define the viral glycoprotein responsible for attachment and cell-cell fusion. Virology **119**:358–371.
14. **Dalgleish, A. G., P. C. Beverley, P. R. Clapham, D. H. Crawford, D. H. Greaves, and R. A. Weiss.** 1984. The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. Nature (London) **312**:763–767.
15. **Frana, M. F., J. N. Behnke, L. S. Sturman, and K. V. Holmes.** 1985. Proteolytic cleavage of the E2 glycoprotein of murine coronavirus: host-dependent differences in proteolytic cleavage and cell fusion. J. Virol. **56**:912–920.
16. **Fuller, S. D., C.-H. von Bonsdorff, and K. Simons.** 1985. Cell surface influenza haemagglutinin can mediate infection by other animal viruses. EMBO J. **4**:2475–2485.
17. **Greve, J. M., G. Davis, A. M. Meyer, C. P. Forte, S. C. Yost, C. W. Marlor, M. E. Kamarck, and A. McClelland.** 1989. The major human rhinovirus receptor is ICAM-1. Cell **56**:839–847.
18. **Gussow, D., and T. Clackson.** 1989. Direct clone characterization from plaques and colonies by the polymerase chain reaction. Nucleic Acids Res. **17**:4000.
19. **Hinoda, Y., M. Neumaier, S. A. Hefta, Z. Drzeniek, C. Wagener, L. Shively, L. J. Hefta, J. E. Shively, and R. J. Paxton.** 1988. Molecular cloning of a cDNA coding biliary glycoprotein I: primary structure of a glycoprotein immunologically crossreactive with carcinoembryonic antigen. Proc. Natl. Acad. Sci. USA **85**:6959–6963. (Erratum, **86**:1668, 1989.)
20. **Hirano, N., T. Goto, T. Ogawa, K. Ono, T. Murakami, and K. Fujiwara.** 1980. Hydrocephalus in suckling rats infected intracerebrally with mouse hepatitis virus, MHV-A59. Microbiol. Immunol. **24**:825–834.
21. **Holmes, K. V., E. W. Doller, and L. J. Sturman.** 1981. Tunicamycin resistant glycosylation of a coronavirus glycoprotein. Virology **115**:334–344.
21a. **Hopp, T. P., and K. R. Woods.** 1981. Prediction of protein antigenic determinants from amino acid sequences. Proc. Natl. Acad. Sci. USA **78**:3824–3828.
22. **Huang, J. Q., C. Turbide, E. Daniels, S. Jothy, and N. Beauchemin.** 1990. Spatiotemporal expression of murine carcinoembryonic antigen (CEA) gene family members during mouse embryogenesis. Development **110**:573–588.
23. **Kessler, M., O. Acuto, C. Storelli, H. Murer, M. Muller, and G. Gemenza.** 1978. A modified procedure for the rapid preparation of efficiently transporting vesicles from small intestine brush border membranes. Their use to investigate some properties of D-glucose and choline transport systems. Biochim. Biophys. Acta **506**:136–154.
24. **Klatzmann, D., E. Champagne, S. Chamaret, J. Gruest, D. Guetard, T. Hercend, J. C. Gluckman, and L. Montagnier.** 1984. T-lymphocyte T4 molecule behaves as the receptor for human retrovirus LAV. Nature (London) **312**:767–768.
25. **Knobler, R. L., L. A. Tunison, and M. B. Oldstone.** 1984. Host genetic control of mouse hepatitis virus type 4 (JHM strain) replication. I. Restriction of virus amplification and spread in macrophages from resistant mice. J. Gen. Virol. **65**:1543–1548.
26. **Kodelja, V., K. Lucas, S. Barnert, S. von Kleist, J. A. Thompson, and W. Zimmermann.** 1989. Identification of a carcinoembryonic antigen gene family in the rat. Analysis of the N-terminal domains reveals immunoglobulin-like, hypervariable regions. J. Biol. Chem. **264**:6906–6912.
26a. **Kyte, J., and R. F. Doolittle.** 1982. A simple method for displaying the hydropathic character of a protein. J. Mol. Biol. **157**:105–132.
27. **Lentz, T. L.** 1990. The recognition event between virus and host cell receptor: a target for antiviral agents. J. Gen. Virol. **71**:751–766.
28. **Lin, S. H., and G. Guidotti.** 1989. Cloning and expression of a cDNA coding for a rat liver plasma membrane ecto-ATPase. The primary structure of the ecto-ATPase is similar to that of the human biliary glycoprotein I. J. Biol. Chem. **264**:14408–14414.
29. **Lowe, T., J. B. Sharefkin, S. Q. Yang, and C. W. Dieffenbach.** 1990. A computer program for selection of oligonucleotide primers for polymerase chain reactions. Nucleic Acids Res. **18**:1757–1761.
30. **Luytjes, W., P. J. Bredenbeek, A. F. Noten, M. C. Horzinek, and W. J. Spaan.** 1988. Sequence of mouse hepatitis virus A59 mRNA 2: indications for RNA recombination between coronaviruses and influenza C virus. Virology **166**:415–422.
31. **Maddon, P. J., A. G. Dalgleish, J. S. McDougal, P. R. Clapham, R. A. Weiss, and R. Axel.** 1986. The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. Cell **47**:333–348.
32. **Mendelsohn, C. L., E. Wimmer, and V. R. Racaniello.** 1989. Cellular receptor for poliovirus: molecular cloning, nucleotide sequence, and expression of a new member of the immunoglobulin superfamily. Cell **56**:855–865.
33. **Ryu, S. E., P. D. Kwong, A. Truneh, T. G. Porter, J. Arthos, M. Rosenberg, X. P. Dai, N. H. Xuong, R. Axel, R. W. Sweet, and W. A. Hendrickson.** 1990. Crystal structure of an HIV-binding recombinant fragment of human CD4. Nature (London) **348**:419–426.
34. **Saiki, R. K., D. H. Gelfand, S. Stoffel, S. J. Scharf, R. Higuchi, G. T. Horn, K. B. Mullis, and H. A. Erlich.** 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science **239**:487–491.
35. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA **74**:5463–5467.
36. **Sattentau, Q. J., and R. A. Weiss.** 1988. The CD4 antigen: physiological ligand and HIV receptor. Cell **52**:631–633.
37. **Shively, J. E., and J. Beatty.** 1985. CEA-related antigens: molecular biology and clinical significance. Crit. Rev. Oncol. Hematol. **2**:355–399.
38. **Smith, A. L., C. B. Cardellichio, D. F. Winograd, M. S. deSouza, S. W. Barthold, and K. V. Holmes.** 1991. Monoclonal antibody to the receptor for murine coronavirus MHV-A59 inhibits virus replication *in vivo*. J. Infect. Dis. **163**:879–882.
39. **Smith, M. S., R. E. Click, and P. G. Plagemann.** 1984. Control of mouse hepatitis virus replication in macrophages by a recessive gene on chromosome 7. J. Immunol. **133**:428–432.
40. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98**:503–517.
41. **Staunton, D. E., M. L. Dustin, H. P. Erickson, and T. A. Springer.** 1990. The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. Cell **61**:243–254. (Erratum, **61**:1157.)
42. **Staunton, D. E., V. J. Merluzzi, R. Rothlein, R. Barton, S. D. Marlin, and T. A. Springer.** 1989. A cell adhesion molecule, ICAM-1, is the major surface receptor for rhinoviruses. Cell **56**:849–853.
43. **Stohlman, S. A., J. A. Frelinger, and L. P. Weiner.** 1980. Resistance to fatal central nervous system disease by mouse hepatitis virus, strain JHM. II. Adherent cell-mediated protection. J. Immunol. **124**:1733–1739.
44. **Sturman, L. S., and K. V. Holmes.** 1977. Characterization of coronavirus. II. Glycoproteins of the viral envelope: tryptic peptide analysis. Virology **77**:650–660.
45. **Sturman, L. S., K. V. Holmes, and J. Behnke.** 1980. Isolation of coronavirus envelope glycoproteins and interaction with the viral nucleocapsid. J. Virol. **33**:449–462.
46. **Sturman, L. S., C. S. Richard, and K. V. Holmes.** 1985. Proteolytic cleavage of the E2 glycoprotein of murine corona-

virus: activation of cell-fusing activity of virions by trypsin and separation of two different 90K cleavage fragments. J. Virol. **56**:904–911.

47. **Sturman, L. S., and K. K. Takemoto.** 1972. Enhanced growth of a murine coronavirus in transformed cells. Infect. Immun. **6**:501–507.
48. **Thomas, P. S.** 1980. Hybridization of denatured RNA and small DNA fragments transferred to nitrocellulose. Proc. Natl. Acad. Sci. USA **77**:5201–5205.
49. **Tomassini, J. E., D. Graham, C. M. De Witt, D. W. Lineberger, J. A. Rodkey, and R. J. Colonno.** 1989. cDNA cloning reveals that the major group rhinovirus receptor on HeLa cells is intercellular adhesion molecule 1. Proc. Natl. Acad. Sci. USA **86**:4907–4911.
50. **Turbide, C., M. Rojas, C. P. Stanners, and N. Beauchemin.** 1991. A mouse carcinoembryonic antigen gene family member is a calcium-dependent cell adhesion molecule. J. Biol. Chem. **266**:309–315.
51. **Vlasak, R., W. Luytjes, W. Spaan, and P. Palese.** 1988. Human and bovine coronaviruses recognize sialic acid-containing receptors similar to those of influenza C viruses. Proc. Natl. Acad. Sci. USA **85**:4526–4529.
52. **Wang, J. H., Y. W. Yan, T. P. Garrett, J. H. Liu, D. W. Rodgers, R. L. Garlick, G. E. Tarr, Y. Husain, E. L. Reinherz, and S. C. Harrison.** 1990. Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. Nature (London) **348**:411–418.
53. **Wege, H., S. Siddell, and V. ter Meulen.** 1982. The biology and pathogenesis of coronaviruses. Curr. Top. Microbiol. Immunol. **99**:165–200.
54. **Weismiller, D. G., L. S. Sturman, M. J. Buchmeier, J. O. Fleming, and K. V. Holmes.** 1990. Monoclonal antibodies to the peplomer glycoprotein of coronavirus mouse hepatitis virus identify two subunits and detect a conformational change in the subunit released under mild alkaline conditions. J. Virol. **64**: 3051–3055.
55. **White, J. M., and D. R. Littman.** 1989. Viral receptors of the immunoglobulin superfamily. Cell **56**:725–728.
56. **Wickens, M., and P. Stephenson.** 1984. Role of the conserved AAUAAA sequence: four AAUAAA point mutants prevent messenger RNA 3′ end formation. Science **226**:1045–1051.
57. **Williams, A. F., and A. N. Barclay.** 1988. The immunoglobulin superfamily-domains for cell surface recognition. Annu. Rev. Immunol. **6**:381–405.
58. **Williams, R. K., G.-S. Jiang, and K. V. Holmes.** 1991. Receptor for mouse hepatitis virus is a member of the carcinoembryonic antigen family of glycoproteins. Proc. Natl. Acad. Sci. USA **88**:5533–5536.
59. **Williams, R. K., G.-S. Jiang, S. W. Snyder, M. F. Frana, and K. V. Holmes.** 1990. Purification of the 110-kilodalton glycoprotein receptor for mouse hepatitis virus (MHV)-A59 from mouse liver and identification of a nonfunctional, homologous protein in MHV-resistant SJL/J mice. J. Virol. **64**:3817–3823.
60. **Yachi, A., and J. E. Shively (ed.).** 1989. The carcinoembryonic gene family. Elsevier Science Publishers BV, New York.